USGS
science for a changing world

In Reply Refer To:
Mail Stop 412 June 10, 2005

## Office of Water Quality Technical Memorandum 2005.02

Subject: **Field Methods**-- Guidance for microbiological monitoring and commercial sources for microbiological media; bacteria kits discontinued on June 17, 2005

### *Purpose of this memorandum*

This memorandum announces that "bacteria kits" currently available through U.S. Geological Survey (USGS) One-Stop Shopping will be discontinued on June 17, 2005. It also provides guidance to USGS personnel on the types of microorganisms that should be monitored in ground and surface water, and provides recommendations for the types of media that should be used for each microorganism and water type according to recommendations by regulatory agencies (U.S. Environmental Protection Agency and Food and Drug Administration). In addition, this memorandum updates Chapter 7, sections 7.1.3 and 7.1.5 of the National Field Manual for the Collection of Water-Quality Data (Myers and Wilde, 2003). Ohio Water Microbiology Laboratory (OWML) personnel investigated several commercial sources for each type of media and, on the basis of formulation, amount of quality control, and price, have suggested appropriate sources for use by the USGS. A brief history describes the groups of microorganisms typically analyzed and the types of media used for each. Following the history, recommendations are made regarding which organisms to culture, the media to use, and the commercial supplier from whom to purchase the media. Contact information for commercial suppliers is provided. Appendixes A–C include the following information: (A) instructions to prepare different types of media from dehydrated ingredients, (B) instructions for quality control, and (C) commercial supplier information for each media type in the form of dehydrated media and pre-poured plates.

***Note:*** The pre-poured plates are 60x15mm in size and do not fit in the older, blue Millipore heater block incubators. BD Biosciences, the manufacturer of the MI, mEI, and modified mTEC pre-poured plates, is working on making smaller plates available in the future. The plates do fit in the newer Millipore single- or dual-chamber incubators.

### *Brief History*

The **total coliform** group includes several genera of bacteria that are found in the human intestines, including the genera *Escherichia*, *Citrobacter*, *Klebsiella*, and *Enterobacter*. Some genera in this group have been found to come from soils, vegetation, and industrial wastes. Total coliforms are commonly used to assess drinking-water and ground-water quality and are used as a screen for fecal contamination. The membrane-filtration procedure using mENDO medium has been used in the past for the enumeration of total coliforms in water (American Public Health Association and others, 1998, p. 9-58). More recently, MI medium was developed by the U.S. Environmental Protection Agency (USEPA) for the simultaneous enumeration of total coliforms and *Escherichia coli* (*E. coli)* from water. The MI medium is able to recover more total coliforms than mENDO medium, with greatly reduced background counts (Brenner and others, 1993).

Another method used to simultaneously enumerate total coliforms and *E. coli* is the Colilert™ method by IDEXX Laboratories, Inc. This method is also approved by the USEPA and is a most-probable-number method, in which a statistical table is used to estimate bacterial concentrations.

The **fecal coliform** group is a subset of the total coliform group and includes thermotolerant coliforms that can grow at elevated temperatures. The fecal coliform group includes bacteria from the following genera: *Escherichia*, *Citrobacter*, and *Klebsiella*. Fecal coliforms were recommended by the USEPA in 1976 as indicators of recreational water quality (U.S. Environmental Protection Agency, 1976). The mFC medium was recommended for use for the enumeration of fecal coliforms in water. Fecal coliforms, however, have been shown to come from non-fecal sources as well (U.S. Environmental Protection Agency, 1986). The USEPA conducted epidemiological studies that showed *E. coli* has a higher correlation with swimming-associated gastroenteritis in freshwater than fecal coliforms (U.S. Environmental Protection Agency, 1986). ***E. coli***, one species in the fecal coliform group, is a natural inhabitant of the gastrointestinal tract of warm-blooded animals and is direct evidence of fecal contamination.

**Fecal streptococci** are another group of bacteria that are used as fecal indicators. Although they are commonly found in feces from warm-blooded animals, a few species are not exclusive to animals (American Public Health Association, 1998, p. 9-74). The membrane-filtration procedure using KF medium has been shown to give a false-positive rate ranging from 10 to 90 percent in marine and fresh waters (American Public Health Association, 1998, p. 9-74-75). **Enterococci** are a subgroup of fecal streptococci and are considered to be a more specific indicator of fecal contamination. Based on the epidemiological studies conducted by the USEPA, enterococci and *E. coli* were found to be equally reliable for indicating gastroinestinal health risk in freshwater (U.S. Environmental Protection Agency, 1986). In marine water, only enterococci provided a strong correlation with swimming-associated gastroenteritis.

In 1986, the USEPA recommended the use of *E. coli* (freshwater only) and enterococci (fresh and marine waters) as indicator organisms of recreational water quality (U.S. Environmental Protection Agency, 1986). At that time, mTEC medium was recommended for enumeration of *E. coli* and mE/EIA medium for enterococci (U.S. Environmental Protection Agency, 1986). In a recent publication (U.S. Environmental Protection Agency, 2000), the mTEC and mE/EIA media have been improved, allowing for faster and easier enumeration of the target bacteria. The USEPA now recommends the use of modified mTEC medium for enumeration of *E. coli* and mEI medium for enterococci.

### *Recommendations for monitoring*

Based on the information above, it is now recommended that, for monitoring ground water, USGS personnel use either the MI method or the Colilert® method for the simultaneous enumeration of total coliforms and *E. coli*. The mEI medium should be used to monitor ground water for enterococci. For surface water, it is recommended that USGS personnel monitor for *E. coli* in freshwater using modified mTEC medium or monitor for enterococci in freshwater and marine water using mEI medium. Colilert® and Enterolert™ may also be used for analyzing surface water, especially for water that is highly turbid or for analyzing sediment samples, which tend to clog membrane filters easily and mask the growth of target colonies. For shellfish-growing/marine waters, mFC medium may be used for monitoring for fecal coliforms and mEI or Enterolert™ may be used for monitoring for enterococci.

| Indicator | Source water | Media |
|---|---|---|
| Total coliform | Ground water | MI or Colilert® |
| E. coli | Ground water | MI or Colilert® |
| Enterococci | Ground water<br>Surface water<br>Marine water | mEI or Enterolert™ |
| E. coli | Surface water | Modified mTEC or Colilert® |
| Fecal coliform | Shellfish-growing waters | mFC |

### *Changes in field supplies*

In recent years, quality-assured pre-poured media plates and dehydrated media have become readily available. Because of this and in order to upgrade the techniques being used, the Office of Water Quality has decided that production and sales of the media kits by the National Field Supply Service (NFSS) at the National Water Quality Laboratory (NWQL), currently sold through One-Stop Shopping, will be discontinued. To give offices time to transition to commercial media, the NFSS kits will continue to be sold through June 17, 2005. After that date, the NFSS and the USGS One Stop Shopping will no longer supply the kits, and field personnel will be responsible for either making their own media from dehydrated ingredients or purchasing pre-poured plates from recommended commercial sources (see Appendix C). Field personnel will also be responsible for performing adequate quality control (QC) (see Appendix B). Field sample collection techniques remain unchanged (Myers and Wilde, 2003).

Making plates from dehydrated media sometimes requires an analytical balance with accuracy of at least 0.01 gram and, in some cases, an autoclave for sterilization. Media made from dehydrated ingredients must be quality controlled prior to use. Instructions for preparation of the different types of media from dehydrated ingredients are found in Appendix A. Instructions for QC of media prepared from dehydrated ingredients can be found in Appendix B. A summary of commercial supplier information for each media type is in Appendix C.

**Always read and understand all Material Safety Data Sheets (MSDS) associated with all chemicals to be handled before their use.**

Pre-poured plates can be purchased if equipment is not available to make media from dehydrated ingredients or if time is limited. Pre-poured plates are already QC'ed by the manufacturer; they do not require mixing of ingredients in a kit, sterilization, or purchasing and pouring into plates. However, some QC of pre-poured plates is still required, although at a reduced frequency as compared to dehydrated media. It is recommended that the pre-poured plates be QC'ed at the beginning and middle of the sampling period, and when the lot number of the plates has changed. Instructions for QC of pre-poured plates can be found in Appendix B.

### *Media*

**mENDO medium** (American Public Health Association and others, 1998, p. 9-58)

mENDO medium is used for the enumeration of total coliforms. **It is recommended that USGS personnel phase out the use of this medium within 1 year from the date of this memorandum and replace it with MI media or the Colilert™ method.**

Dehydrated mENDO medium can be purchased from Fisher Scientific. The current cost for 500 grams (catalog no. DF0736172) is $92.52. The cost for 100 grams (catalog no. DF0736154) is $41.14. If the medium is made from dehydrated ingredients, 95 percent denatured ethanol also must be purchased. It can be purchased from Fisher Scientific in a quantity of 4 liters (catalog no. A405P-4) for $30.54.

Pre-poured plates can be purchased from Hardy Diagnostics. The cost for 1 plate (catalog no. G128) is $0.73. If pre-poured plates are purchased, 95% ethanol is not needed.

**MI medium** (U.S. Environmental Protection Agency, 2002)

MI medium is used for the simultaneous enumeration of *E. coli* and total coliforms in ground water. It is recommended that MI medium replace the mENDO medium.

Dehydrated MI medium can be purchased from Government Scientific Source. The current cost for 500 grams (catalog no. 214883) is $1275.00. The cost for 100 grams (catalog no. 214882) is $255.00. Cefsulodin must be purchased separately if the medium is made from dehydrated ingredients. It can be purchased from Sigma-Aldrich in a quantity of 100 milligrams (catalog no. C-8145) for $24.00. If MI medium is made from dehydrated stocks, the user must have an analytical balance with accuracy of at least 0.01 gram.

Pre-poured plates can be purchased from Microbiology International. The cost for 20 plates (catalog no. B14986) is $58.45. The cost for 100 plates (catalog no. B14985) is $313.00. Cefsulodin is incorporated in the pre-poured plates and does not have to be added.

**mFC medium** (American Public Health Association and others, 1998, p. 9-63)

mFC medium is used for the enumeration of fecal coliforms. **It is recommended that USGS personnel phase out the use of mFC medium (and the use of fecal coliforms as indicator organisms) when possible and replace it with another medium and indicator organism—either modified mTEC medium for *E. coli* or mEI medium for enterococci.** In some cases, it may not be possible to phase out mFC medium and fecal coliforms because they are still used for historical data comparisons and for monitoring drinking water, wastewater effluents, and shellfish-growing waters.

Dehydrated mFC medium can be purchased from Fisher Scientific. The cost for 500 grams (catalog no. DF0677-17-3) is $82.92. The cost for 100 grams (catalog no. DF0677-15-5) is $36.53. Rosolic acid must also be purchased if the medium is made from dehydrated ingredients. It can be purchased from Fisher Scientific in packs of 6 (1 gram each) (catalog no. DF3228-09-1) for $53.85. The rosolic acid must be dissolved in 0.2 N NaOH, which may be purchased from Fisher. The cost for 500 milliliters NaOH is $9.90 (catalog no. AC34968-5000).

Pre-poured plates can be purchased from Hardy Diagnostics. The cost for 1 plate (catalog no. G126) is $0.98. Rosolic acid is incorporated in the pre-poured plates and does not have to be added.

**KF medium** (Britton and Greeson, 1989)

KF medium is used for the enumeration of fecal streptococci. **It is recommended that USGS personnel phase out the use of KF medium within 1 year of the date of this memorandum and replace it with mEI medium for enterococci.**

Dehydrated KF medium can be purchased from Hach Company. The cost for 500 grams (catalog no. 1485334) is $98.05. If the medium is made from dehydrated ingredients, 1 percent pre-sterilized TTC must also be purchased. TTC can be purchased from Hach in a quantity of 100 milliliters (catalog no. 24060-42) for $24.50.

Pre-poured plates can be purchased from Schleicher & Schuell (S&S). The cost for 10 plates (catalog no. P1352) is $26.79 (minimum order of 3 packs). TTC is incorporated in the pre-poured plates and does not have to be added.

**mTEC medium** (U.S. Environmental Protection Agency, 2000)

mTEC medium is used for the enumeration of *E. coli*. It is recommended that USGS personnel phase out the use of mTEC medium and switch to modified mTEC medium. The modified mTEC method does not require transfer of the membrane filter to a urea-phenol substrate, and the colonies on modified mTEC are easier to enumerate than on the original mTEC. The USEPA recommends the use of modified mTEC medium over the original mTEC medium for the enumeration of *E. coli* from water.

Dehydrated mTEC medium can be purchased from S&S. The cost for 500 grams (catalog no. 74217A) is $64.18.

Pre-poured plates can be purchased from S&S. The cost for 10 plates (catalog no. P7300) is $11.07 (minimum order of 3 packs).

Phenol red and urea must also be purchased whether the medium is being made from dehydrated ingredients or if pre-poured plates are purchased. Phenol red can be purchased from Fisher Scientific (catalog no. AC41724-0050; 5 grams for $12.26). Urea can be purchased from Fisher Scientific (catalog no. U15-500; 500 grams for $17.19).

**Modified mTEC medium** (U.S. Environmental Protection Agency, 2000)

Modified mTEC medium is recommended by the USEPA for the enumeration of *E. coli* from ambient waters. Modified mTEC medium is an improvement over the original mTEC medium as plates are easier to read and the additional incubation on a urea-phenol substrate is not needed.

Dehydrated modified mTEC medium can be purchased from Government Scientific Source. The cost for 100 grams (catalog no. 214884) is $309.96. The cost for 500 grams (catalog no. 214880) is $1,343.00.

Pre-poured plates can be purchased from Microbiology International. The cost for 20 plates (catalog no. B15044) is $68.39. The cost for 100 plates (catalog no. B15046) is $338.18.

**mEI medium** (U.S. Environmental Protection Agency, 2000)

mEI medium is recommended by the USEPA for the enumeration of enterococci.

Dehydrated mEI medium can be purchased from Government Scientific Source. The cost for 500 g (catalog no. 214881) is $1343.00. The cost for 100 g (catalog no. 214885) is $309.96. Indoxyl β-D-glucoside, nalidixic acid, and 1% presterilized TTC must also be purchased if the medium is made from dehydrated ingredients. Indoxyl β-D-glucoside can be purchased from Sigma-Aldrich Corp. in a quantity of 2 g (catalog no. I3750-2G) for $822 or in a quantity of 1 g (catalog no. I3750-1G) for $456.50. Nalidixic acid can be purchased from Hach in a quantity of 25 g (catalog no. 24071-24) for $54.50. Preparation of a nalidixic acid solution requires it to be dissolved in 10 N NaOH, which may be purchased from Fisher. The cost for 1 liter is $37.05 (catalog no. SS255-1). The 1 percent presterilized TTC can be purchased from Hach in a quantity of 100 mL (catalog no. 24060-42) for $24.50.

Pre-poured plates can be purchased from Microbiology International. The cost for 20 plates (catalog no. B15045) is $68.39, and the cost for 100 plates (catalog no. B15047) is $325.65.

**References**

American Public Health Association, American Water Works Association, and Water Environment Federation, 1998, Standard methods for the examination of water and wastewater (20th ed.): Washington, D.C., American Public Health Association, p. 9-1 to 9-75.

Brenner, K.P., Rankin, C.C., Roybal, Y.R., Stelma Jr., G.N., Scarpino, P.V., and Dufour, A.P., 1993, New medium for the simultaneous detection of total coliforms and Escherichia coli in water: Applied and Environmental Microbiology, v. 59, no. 11, p. 3534-3544.

Britton, L.J., and Greeson, P.E., eds., 1989, Methods for collection and analysis of aquatic biological and microbiological samples: U.S. Geological Survey Techniques of Water-Resources Investigations, book 5, chap. A4, 363 p.

Myers, D.N., and Wilde, F.D., eds., November 2003, Biological indicators (3d ed.): U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A7, accessed March 16, 2005 at http://pubs.water.usgs.gov/twri9A7/

U.S. Environmental Protection Agency, 1976, Fecal coliform bacteria, *in* Quality criteria for water: Washington, D.C., Office of Water and Hazardous Materials, p. 42-50.

U.S. Environmental Protection Agency, 1986, Ambient water quality criteria for bacteria-1986: Washington, D.C., Office of Water Regulations and Standards, Criteria and Standards Division, EPA-440/5-84/002.

U.S. Environmental Protection Agency, 2000, Improved enumeration methods for the recreational water quality indicators: Enterococci and *Escherichia coli*: Washington, D.C., EPA/821/R-97/004, 49 p.

U.S. Environmental Protection Agency, 2002, Method 1604: Total coliforms and *Escherichia coli* in water by membrane filtration using a simultaneous detection technique (MI medium): Washington, D.C., EPA 821-R-02-024, 14 p.

Timothy L. Miller /s/
Chief, Office of Water Quality

This memorandum replaces information found in Chapter 7, sections 7.1.3 and 7.1.5, of the National Field Manual for the Collection of Water-Quality Data (Myers, D.N., and Wilde, F.D., eds., November 2003, Biological indicators (3d ed.): U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A7. Updates will be published on the web site at http://pubs.water.usgs.gov/twri9A7/.

***Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.***

Distribution: All WRD Employees
Attachments
**********

Timothy L. Miller
Chief, Office of Water Quality
U.S. Geological Survey
412 National Center
Reston, VA 20192
703/648-6868
tlmiller@usgs.gov

## Appendix A – Instructions for Media Preparation from Dehydrated Ingredients

**Instructions for preparation of mENDO medium** (American Public Health Association and others, 1998, p. 9-58)

NOTE: It is not recommended that this medium be made up in large quantities and stored in the refrigerator; therefore, the instructions to prepare only 100 mL of media are given. Plates should be poured immediately and used within 5 days.

a. Prepare 100 mL of a 2% ethanol solution by combining 2 mL of 95% denatured ethanol with 98 mL of deionized or distilled water. Mix well.
b. Combine 5.1 g of dehydrated mENDO medium with 100 mL of the 2% ethanol solution in a 250-mL flask.
c. Stir the mixture well for several minutes to break up clumps and prevent medium from adhering to the flask.
d. Place the flask in a heated water bath or on a hot plate and heat slowly to boiling. If using a hot plate, stir the mixture constantly or use a stir bar and magnetic stirring hot plate to prevent scorching. **Do not autoclave.**
e. When the medium approaches the boiling point, promptly remove from heat. **Do not boil.**
f. Cool the medium to a temperature of about 45-50°C and pour 6 to 7 mL in 50-mm Petri-dish bottoms. Quickly place Petri-dish tops loosely on Petri-dish bottoms to allow condensation to escape.
g. When the medium has solidified (about 10 minutes), close the Petri dishes by pressing firmly on the tops. These plates are suitable for use after the medium has solidified. About 15 to 20 plates can be prepared from 100 mL of medium. Label and date.
h. Prepared Petri dishes sealed in small plastic bags to prevent drying and stored in the darkness of a refrigerator, can be used **for a maximum of 5 days.**

**Instructions for preparation of MI medium** (U.S. Environmental Protection Agency, 2002)

| Ingredients | To make 100 mL | To make 1 L |
|---|---|---|
| Dehydrated MI agar | 3.65 g | 36.52 g |
| Deionized or distilled water | 100 mL | 1000 mL |

a. Add amounts specified in above table of dehydrated MI agar to deionized or distilled water in the appropriate-sized flask.
b. Stir this mixture for several minutes to break up clumps. Make sure that none of the medium adheres to the bottom or side of the flask.
c. Place the flask in a heated water bath or on a hot plate and heat slowly to boiling. If using a hot plate, stir the mixture constantly or use a stir bar and magnetic stirring hot plate to prevent scorching. After boiling begins, remove the flask from the heat source.
d. Aliquot 100-mL volumes into autoclavable bottles and autoclave at 121°C and 15 $lb/in^2$ pressure for 15 minutes. Allow to cool to 45-50°C. Either pour plates (step e) or store 100-mL aliquots of medium at 4°C (step f).
e. Pouring plates:
    - Preparation for pouring:
        1. Prepare cefsulodin solution by adding 0.02 g of cefsulodin to 20 mL of deionized or distilled water. Sterilize the cefsulodin solution by filtering through a disposable, sterile 0.22-µm syringe filter. Store in a sterile container at 4°C until needed. Prepare a fresh solution each time MI is made. Do not save any unused portion because it will degrade.
        2. **Cefsulodin solution is added after the MI medium is autoclaved and cooled to 45-50°C.**
        3. Add 0.5 mL of freshly prepared cefsulodin solution to each 100-mL volume of tempered agar medium and mix gently.
    - Pour 6 to 7 mL of the medium into 50-mm Petri-dish bottoms. Quickly place the Petri-dish tops loosely onto the bottoms to allow condensation to escape.
    - When the medium has solidified (about 10 minutes), close the Petri dishes by pressing firmly on the tops. These plates are suitable for use after the medium has solidified. About 15 to 20 plates can be prepared from 100 mL of medium. Label and date.
    - Prepared Petri dishes, sealed in small plastic bags to prevent drying, can be **stored in a refrigerator for up to 2 weeks.**
f. Long-term storage:
    - 100-mL aliquots of MI medium without cefsoludin can be **stored at 4°C for up to 6 months.**
    - To prepare plates from refrigerated agar, melt the medium using a beaker with water on a hot plate or by placing in the autoclave for a 5-minute cycle. Add cefsulodin solution as indicated in step e and follow above instructions to pour plates.

**Instructions for preparation of mFC medium** (American Public Health Association and others, 1998, p. 9-63)

NOTE: It is not recommended that this medium be made up in large quantities and stored in the refrigerator; therefore, the instructions to prepare only 100 mL of media are given. Plates should be poured immediately and used within 72 hours.

a. Use purchased 0.2 N NaOH or prepare a 0.2 *N* NaOH (sodium hydroxide) solution by dissolving 8.0 g of NaOH in deionized or distilled water and bringing the volume up to 1 L.
b. Prepare a rosolic acid solution by adding 0.1 g of rosolic acid crystals to 10 mL of 0.2 *N* NaOH. Shake the mixture to dissolve crystals. **Do not heat**. The crystals will dissolve in 15 minutes. Prepare a new solution each time mFC is made. Do not save any unused portion.
c. Combine 5.2 g of dehydrated mFC medium with 100 mL of deionized or distilled water into a 250-mL flask.
d. Stir the mixture well for several minutes to break up clumps and prevent medium from adhering to the flask.
e. Place the flask in a heated water bath or on a hot plate and heat slowly to 90°C. If using a hot plate, stir the mixture constantly or use a stir bar and magnetic stirring hot plate to prevent scorching. **Do not autoclave.**
f. With a clean pipette, add 1 mL of rosolic acid solution per 100 mL of medium. Continue heating for a maximum of 1 minute; then remove from the heat.
g. Cool the medium to a temperature of about 45-50°C and pour 6 to 7 mL in 50-mm Petri-dish bottoms. Quickly place Petri-dish tops loosely on Petri-dish bottoms to allow condensation to escape.
h. When the medium has solidified (about 10 minutes), close the Petri dishes by pressing firmly on the tops. These plates are suitable for use after the medium has solidified. About 15 to 20 plates can be prepared from 100 mL of medium. Label and date.
i. Prepared Petri dishes, sealed in small plastic bags to prevent drying, can be **stored in a refrigerator for up to 72 hours.**

**Instructions for preparation of KF medium** (Britton and Greeson, 1989)

NOTE: It is not recommended that this medium be made up in large quantities and stored in the refrigerator; therefore, the instructions to prepare only 100 mL of media are given. Plates should be poured immediately and used within 2 weeks.

a. Combine 7.64 g of dehydrated KF medium with 100 mL of deionized or distilled water into a 250-mL flask.
b. Stir the mixture well for several minutes to break up clumps and prevent medium from adhering to the flask.
c. Place the flask in a heated water bath or on a hot plate and heat slowly to boiling. If using a hot plate, stir the mixture constantly or use a stir bar and magnetic stirring hot plate to prevent scorching.
d. After boiling begins, continue heating for 5 minutes. Remove from heat and cool to 45-50°C. **Do not autoclave.**
e. Aseptically add 1 mL of commercially available pre-sterilized 1% TTC (triphenyltetrazolium chloride) solution to 100 mL of the medium and mix well.
f. Pour 6 to 7 mL in 50-mm Petri-dish bottoms. Quickly place Petri-dish tops loosely on Petri-dish bottoms to allow condensation to escape.
g. When the medium has solidified (about 10 minutes), close the Petri dishes by pressing firmly on the tops. These plates are suitable for use after the medium has solidified. About 15 to 20 plates can be prepared from 100 mL of medium. Label and date.
h. Prepared Petri dishes sealed in small plastic bags to prevent drying can be **stored in a refrigerator for up to 2 weeks if a sterile TTC solution is used.**

**Instructions for preparation of mTEC medium** (U.S. Environmental Protection Agency, 2000)

| Ingredients | To make 100 mL | To make 1 L |
|---|---|---|
| Dehydrated mTEC Agar | 4.53 g | 45.3 g |
| Deionized or distilled water | 100 mL | 1000 mL |

a. Add amounts specified in above table of dehydrated mTEC agar to deionized or distilled water in the appropriate-sized flask.
b. Stir this mixture for several minutes to break up clumps. Make sure that none of the medium adheres to the bottom or side of the flask.
c. Place the flask in a heated water bath or on a hot plate and heat slowly to boiling. If using a hot plate, stir the mixture constantly or use a stir bar and magnetic stirring hot plate to prevent scorching. After boiling begins, remove the flask from the heat source.
d. Aliquot 100-mL volumes into autoclavable bottles and autoclave at 121°C and 15 $lb/in^2$ pressure for 15 minutes. Allow to cool to 45-50°C. Either pour plates (step e) or store 100-mL aliquots of medium at 4°C (step f).
e. Pouring plates:
    - Pour 6 to 7 mL of the medium into 50-mm Petri-dish bottoms. Quickly place the Petri-dish tops loosely onto the bottoms to allow condensation to escape.
    - When the medium has solidified (about 10 minutes), close the Petri dishes by pressing firmly on the tops. These plates are suitable for use after the medium has solidified. About 15 to 20 plates can be prepared from 100 mL of medium. Label and date.
    - Prepared Petri dishes, sealed in small plastic bags to prevent drying, can be **stored in a refrigerator for up to 2 weeks.**
f. Long-term storage:
    - 100-mL aliquots of mTEC medium can be **stored at 4°C for up to 6 months.**
    - To prepare plates from refrigerated agar, melt the medium using a beaker with water on a hot plate or by placing in the autoclave for a 5-minute cycle. Follow above instructions to pour plates (step e).

**Instructions for preparation of urea-phenol broth**

| Ingredients | Amount |
|---|---|
| Urea | 2.0 g |
| Phenol red | 0.01 g |
| Deionized or distilled water | 100 mL |

a. Combine ingredients into a 250-mL flask and mix for several minutes with a stir bar. Although all of the phenol red crystals may not dissolve, the solution is nevertheless acceptable to use.
b. Adjust pH of urea-phenol broth to 3-4 with a few drops of 1 *N* HCl (hydrochloric acid). The substrate solution is a straw-yellow color at this pH.
c. Urea-phenol broth can be **stored in the refrigerator for up to 2 weeks**, as long as the solution remains straw yellow in color.

**Instructions for preparation of modified mTEC medium** (U.S. Environmental Protection Agency, 2000)

| Ingredients | To make 100 mL | To make 1 L |
|---|---|---|
| Dehydrated modified mTEC Agar | 4.56 g | 45.6 g |
| Deionized or distilled water | 100 mL | 1000 mL |

a. Add amounts specified in above table of dehydrated modified mTEC agar to deionized or distilled water in the appropriate-sized flask.
b. Stir this mixture for several minutes to break up clumps. Make sure that none of the medium adheres to the bottom or side of the flask.
c. Place the flask in a heated water bath or on a hot plate and heat slowly to boiling. If using a hot plate, stir the mixture constantly or use a stir bar and magnetic stirring hot plate to prevent scorching. After boiling begins, remove the flask from the heat source.
d. Aliquot 100-mL volumes into autoclavable bottles and autoclave at 121°C and 15 $lb/in^2$ pressure for 15 minutes. Allow to cool to 45-50°C. Either pour plates (step e) or store 100-mL aliquots of medium at 4°C (step f).
e. Pouring plates:
    - Pour 6 to 7 mL of the medium into 50-mm Petri-dish bottoms. Quickly place the Petri-dish tops loosely onto the bottoms to allow condensation to escape.
    - When the medium has solidified (about 10 minutes), close the Petri dishes by pressing firmly on the tops. These plates are suitable for use after the medium has solidified. About 15 to 20 plates can be prepared from 100 mL of medium. Label and date.
    - Prepared Petri dishes, sealed in small plastic bags to prevent drying, can be **stored in a refrigerator for up to 2 weeks.**
f. Long-term storage:
    - 100-ml aliquots of modified mTEC medium can be **stored at 4°C for up to 6 months.**
    - To prepare plates from refrigerated agar, melt the medium using a beaker with water on a hot plate or by placing in the autoclave for a 5-minute cycle. Follow above instructions to pour plates (step e).

**Instructions for preparation of mEI medium** (U.S. Environmental Protection Agency, 2000)

| Ingredients | To make 100 mL | To make 1 L |
|---|---|---|
| Dehydrated mEI agar | 7.12 g | 71.2 g |
| Indoxyl ß-D-glucoside | 0.075 g | 0.75 g |
| Deionized or distilled water | 100 mL | 1000 mL |

**a.** Add amounts of ingredients specified in above table in the appropriate-sized flask.
**b.** Stir this mixture for several minutes to break up clumps. Make sure that none of the medium adheres to the bottom or side of the flask.
**c.** Place the flask in a heated water bath or on a hot plate and heat slowly until boiling. If using a hot plate, stir the mixture constantly or use a stir bar and magnetic stirring hot plate to prevent scorching. After boiling begins, remove the flask from the heat source.
**d.** Autoclave at 121°C and 15 lb/in$^2$ pressure for 15 minutes. Allow to cool to 45-50°C.
**e.** Prepare a nalidixic acid solution by combining 0.48 g of nalidixic acid with 0.4 mL 10 *N* NaOH (sodium hydroxide) and 10 mL of deionized or distilled water. Mix well. **Do not heat. The 10*N* NaOH is extremely caustic and requires very careful handling.***
**f.** Prepare a TTC (triphenyltetrazolium chloride) solution by combining 0.1 g of TTC to 10 mL of deionized or distilled water. Warm to dissolve. *(Alternatively, purchase a presterilized 1% TTC solution)*
**g.** When the medium cools to 45-50°C, the dissolved reagents may be added (see amounts in table below). If the medium is not going to be used within 24 hours, the nalidixic acid and TTC solutions must be sterilized by aseptically filtering through a disposable, sterile 0.22-µm-pore-size syringe filter.

| Ingredients | To make 100 mL | To make 1 L |
|---|---|---|
| Nalidixic acid solution | 0.52 mL | 5.2 mL |
| TTC solution | 0.2 mL | 2 mL |

****An alternate method for making the nalidixic acid solution is to mix 0.34g of nalidixic acid in 7mL of reagent-grade distilled water, add a few drops of 0.1N NaOH to dissolve. Filter sterilize if the medium is not going to be used w/in 24 hours. Add 5mL to mEI medium when cooled to 45-50 degrees C.***

**h.** Aliquot 100-mL volumes into sterile containers for storage at 4°C or pour 6 to 7 mL of the medium into 50-mm Petri-dish bottoms. Quickly place the Petri-dish tops loosely onto the bottoms to allow condensation to escape.
**i.** When the medium has solidified (about 10 minutes), close the Petri dishes by pressing firmly on the tops. These plates are suitable for use after the medium has solidified. About 15 to 20 plates can be prepared from 100 mL of medium. Label and date.
**j.** Prepared Petri dishes, sealed in small plastic bags to prevent drying, can be **stored in a refrigerator for up to 2 weeks.**
**k.** 100-ml aliquots of mEI medium can be **stored at 4°C for up to 6 months.**
**l.** To prepare plates from refrigerated agar, melt the medium using a beaker with water on a hot plate. **Do not autoclave the medium once the nalidixic acid and TTC solutions have been added.** Follow above instructions to pour plates (steps h-j).

**References:**

American Public Health Association, American Water Works Association, and Water Environment Federation, 1998, Standard methods for the examination of water and wastewater (20th ed.): Washington, D.C., American Public Health Association, p. 9-1 to 9-73.

Britton, L.J., and Greeson, P.E., eds., 1989, Methods for collection and analysis of aquatic biological and microbiological samples: U.S. Geological Survey Techniques of Water-Resources Investigations, book 5, chap. A4, 363 p.

U.S. Environmental Protection Agency, 2000, Improved enumeration methods for the recreational water quality indicators: Enterococci and *Escherichia coli*: Washington, D.C., EPA/821/R-97/004, 49 p.

U.S. Environmental Protection Agency, 2002, Method 1604: Total coliforms and *Escherichia coli* in water by membrane filtration using a simultaneous detection technique (MI medium): Washington, D.C., EPA 821-R-02-024, 14 p.

## Appendix B – Quality control instructions for media prepared from dehydrated ingredients and for prepoured plates

Appendix B contains information regarding the positive and negative control organisms that are recommended for quality-control checks of media prepared from dehydrated ingredients and for pre-poured plates. Ordering information for the organisms and instructions on how to perform the quality control are also provided.

[FC, fecal coliform; TC, total coliform; FS, fecal streptococci; NC, non coliform]
Ordering information is for Remel, Inc

| Media type | Positive control | Negative control |
|---|---|---|
| mTEC | *Escherichia coli* (EC) | *Enterobacter cloacae* (TC) |
| Modified mTEC | *Escherichia coli* (EC) | *Enterobacter cloacae* (TC) |
| mFC | *Escherichia coli* (FC) | *Enterobacter cloacae* (TC) |
| mENDO | *Escherichia coli* (TC) | *Pseudomonas aeruginosa* (NC) |
| MI | *Escherichia coli* (EC) and *Enterobacter cloacae* (TC) | *Pseudomonas aeruginosa* (NC) |
| KF | *Enterococcus faecalis* (FS) | *Enterobacter cloacae* (TC) |
| mEI | *Enterococcus faecalis* (FS) | *Enterobacter cloacae* (TC) |

| Organism | Remel Catalog Number | Price | Number of vials (tests) included | Number of organisms per vial |
|---|---|---|---|---|
| *Escherichia coli* strain C1 | 4751985 | $207.25 | 10 | <50 |
| *Enterobacter cloacae* 13047 | 4757090 | $207.25 | 10 | <50 |
| *Enterococcus faecalis* 29212 | 4741009 | $315.90 | 10 | <50 |
| *Pseudomonas aeruginosa* 27853 | 4757060 | $207.25 | 10 | <50 |

**Instructions for performing quality control**

For each batch of media prepared from dehydrated ingredients, three plates should be saved for quality control—filter blank, positive control, and negative control.

Pre-poured plates should also be QC'ed at least at the beginning and middle of the sampling period and when the lot number of the plates has changed. Three plates should be saved for each quality-control test—filter blank, positive control, and negative control.

For the filter blank – run approximately 50 mL of sterile buffer water through the membrane-filtration apparatus with a filter in place to ensure complete sterilization of equipment and buffer. Incubate plate for the prescribed temperature and time and then observe results. There should be no growth on the filter blank.

For the positive control, rehydrate the organism following the manufacturer's instructions. Pour the vial (or two vials if testing MI medium) into a pre-warmed 99-mL dilution blank and plate the entire 100-mL volume using the membrane-filtration technique. Incubate plate for the prescribed temperature and time and then observe results.

For the negative control, rehydrate the organism following the manufacturer's instructions. Pour the vial into a pre-warmed 99-mL dilution blank and plate the entire 100-mL volume using the membrane-filtration technique using a new, sterile filter funnel. Incubate plate for the prescribed temperature and time and then observe results.

**It is very important to document all quality-control results to ensure legally-defensible data.** For media prepared from dehydrated ingredients, record the date the medium was prepared, initials of the preparer and QC analyst, the lot number of the ingredients, the QC organisms and lot numbers, and the results of the quality-control test. For pre-poured plates, record the date the plates were received, the lot number and expiration date of the plates, the QC organisms and lot numbers, initials of the QC analyst, and the results of the quality-control test. If the positive control organisms do not grow, or if the negative control organisms grow, there is a problem with the prepared medium or the control organisms used. The medium should not be used until the problem is identified. Test the plates again with a new set of controls to be sure the error was not in the preparation of the controls. If the same results are obtained, prepare a new batch of medium and perform the quality-control check again. Contact the manufacturers of the medium and the controls if the problems persist.

## Appendix C - Ordering information for dehydrated media, pre-poured plates, and NWQL kits and commercial source information

| | Dehydrated media | | | | Pre-poured plates | | | | NWQL kits | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Media** | **Vendor** | **Catalog no.** | **Quantity** | **Price*** | **Vendor** | **Catalog no.** | **Quantity** | **Price*** | **Catalog no.** | **Quantity** | **Price*** |
| mENDO | Fisher | DF0736154 | 100 g | $ 41.14 | Hardy | G128 | 1 plate | $ 0.73 | Q4BACT | 15 plates | $ 18.09 |
| | | DF0736172 | 500 g | $ 92.52 | | | | | | | |
| 95% ethanol | Fisher | A405P-4 | 4 L | $ 30.54 | | | | | | | |
| MI | GSS | 214882 | 100 g | $ 225.00 | Microbiology International | B14986 | 20 plates | $ 58.45 | NA | | |
| cefsulodin | GSS | 214883 | 500 g | $ 1,275.00 | Microbiology International | B14985 | 100 plates | $ 313.00 | | | |
| | Aldrich | C-8145 | 100 mg | $ 24.00 | | | | | | | |
| mFC | Fisher | DF0677-15-5 | 100 g | $ 36.53 | Hardy | G126 | 1 plate | $ 0.98 | Q2BACT | 15 plates | $ 20.52 |
| | Fisher | DF0677-17-3 | 500 g | $ 82.92 | | | | | | | |
| rosolic acid | Fisher | DF3228-09-1 | 1 g/pack 6 | $ 53.85 | | | | | | | |
| 0.2 N NaOH | Fisher | AC34968-5000 | 500 mL | $ 9.90 | | | | | | | |
| KF | Hach | 1485334 | 500 g | $ 98.05 | S&S | P1352 | 10 plates | $ 26.79 | Q3BACT | 15 plates | $ 23.26 |
| 1% TTC | Hach | 24060-42 | 100 mL | $ 24.50 | | | | | | | |
| mTEC | S&S | 74217A | 500 g | $ 64.18 | S&S | P7300 | 10 plates | $ 11.07 | Q371BACT | 15 plates | $ 25.75 |
| phenol red | Fisher | AC41724-0050 | 5 g | $ 12.26 | | | | | | | |
| urea | Fisher | U15-500 | 500 g | $ 17.19 | | | | | | | |
| modified mTEC | GSS | 214884 | 100 g | $ 309.96 | Microbiology International | B15044 | 20 plates | $ 68.39 | NA | | |
| | GSS | 214880 | 500 g | $ 1,343.00 | Microbiology International | B15046 | 100 plates | $ 338.18 | | | |
| mEI | GSS | 214885 | 100 g | $ 309.96 | Microbiology International | B15045 | 20 plates | $ 68.39 | Q440BACT | 15 plates | $ 74.27 |
| nalidixic acid | GSS | 214881 | 500 g | $ 1,343.00 | Microbiology International | B15047 | 100 plates | $ 325.65 | | | |
| 10 N NaOH | Hach | 24071-24 | 25 g | $ 54.50 | | | | | | | |
| 1% TTC | Fisher | SS255-1 | 1 L | $ 37.05 | | | | | | | |
| | Hach | 24060-42 | 100 mL | $ 24.50 | | | | | | | |

*Prices are from March 2005

**Fisher** Fisher Scientific
**GSS** Government Scientfic Source
**Hach** Hach Company
**S&S** Schleicher & Schuell

**Commercial Sources for media and supplies**

**Aldrich**
P.O. Box 2060
Milwaukee, WI 53201
(800) 325-3010
(800) 325-5052 (fax)
www.sigma-aldrich.com

**Fisher Scientific**
(800) 766-7000
(800) 926-1166 (fax)
www.fishersci.com

**Government Scientific Source, Inc.**
12351 Sunrise Valley Drive
Reston, VA 20191
(800) 248-8030
(703) 734-1803 (fax)
www.govsci.com

**Hach Company**
P.O. Box 389
Loveland, Colorado 80539-0389
(800) 227-4224
(970) 669-2932 (fax)
www.hach.com

**Hardy Diagnostics**
1430 West McCoy Lane
Santa Maria, CA 93455
(800) 266-2222
(805) 346-2760
sales@hardydiagnostics.com (email)
www.hardydiagnostics.com

**IDEXX Laboratories, Inc.**
One IDEXX Drive
Westbrook, Maine 04092
(800) 321-0207
(207) 856-0300
(207) 856-0346 (fax)
www.idexx.com

**Microbiology International**
5108 Pegasus Court, Suite A
Frederick, MD 21704
800/EZ-MICRO (phone)
301/662-8096 (fax)
http://www.800ezmicro.com

**Remel Inc.**
12076 Santa Fe Drive
P.O. Box 14428
Lenexa, KS 66215
(800) 447-3635
(800) 621-8251 (fax)
remel@remel.com (email)
www.remelinc.com

**Schleicher & Schuell (S&S)**
950 Congress Avenue
Riviera Beach, FL 33404
(800) 645-2302
(888) 645-2302 (fax)
MicroScienceUSA@schleicher-schuell.com
(email)
www.schleicher-schuell.com

**Sigma-Aldrich Corp.**
St. Louis, MO, USA
(800) 325-3010
(314) 771-5757 (fax)
OC_DOM_HC@sial.com (email)
www.sigmaaldrich.com